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The Effect of Coronary Artery Ligation at Different Sites on Sympathetic Remodeling Post Myocardial Infarction in Rats

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OBJECTIVES Sympathetic remodeling plays an important role in the initiation and development of ventricular arrhythmia and sudden cardiac death (SCD) secondary to myocardial infarction (MI). The rat model of heart failure post MI is routinely used in the study of sympathetic remodeling post MI. This model generally requires ligating the left anterior descending coronary artery (LAD) quite close to its origin, in order to produce a sufficiently large infarct size to induce discernible heart failure. However, this model is associated with high mortality. Furthermore, severe heart failure may develop confounding effects in the study of the relationship between sympathetic remodeling and cardiac arrhythmia per se. We hypothesize that ligation of the LAD at a more distal site from its origin reduces mortality and produces a similar level of sympathetic remodeling. The objective of this study is to investigate the effect of coronary artery ligation at different sites on sympathetic remodeling post MI in rats.

METHODS LAD of Sprague-Dawley rats was ligated 2mm (conventional site group, CS, n=25) or 6mm (distal site group, DS, n=25) distal to its origin. A sham operated group (SO, n=10, passage of a needle beneath the LAD 2mm distal to its origin and without LAD ligation) was included. The mortality, and histological changes, including the density of tyrosine hydroxylase (TH)-positive nerve fibers, were determined at six weeks post MI induction.

RESULTS Compared with the CS group, in the DS group there was a significant reduction in total mortality (48% vs 12%, $p<0.01$). The reduction in mortality was predominantly due to lesser incidence of ventricular fibrillation within 24 hours post surgery (36% vs 8%, $p<0.05$). Histology confirmed MI in both CS and DS groups. The densities of TH-positive (i.e., mature) nerve fibers were more abundant in the infarct marginal zone of the CS or DS group rats compared with the corresponding zone in the SO group of animals (3589±1332 vs. 3137±775 vs. 1108±356 μm2/mm2 ($p < 0.01$) resp. CS vs. DS vs. SO). The DS and CS groups had similar densities of TH-positive nerve fibers (3589±1332 vs. 3137±775 μm2/mm2, p = NS).

CONCLUSIONS LAD ligation at different sites resulted in similar levels of sympathetic remodeling in the infarct marginal zone at six weeks post MI. These results suggest that MI induced by LAD ligation at a more distal site with lower mortality is suitable for the study of sympathetic remodeling.

GW26-e0377

The Study of Chronic Intermittent Hypoxia Caused by Obstructive Sleep Apnea to the Rat Myocardial Cell Apoptosis and Myocardial Fibrosis

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OBJECTIVES The study of chronic intermittent hypoxia caused by obstructive sleep apnea to the rat myocardial cell apoptosis and myocardial fibrosis.

METHODS (1) Rats were randomly divided into chronic intermittent hypoxia group (CIH) and control group (NC) : chronic intermittent hypoxia rats group (CIH) were risen into plexiglass chambers which were supplied by chronic intermittent hypoxia in 8 hours a day, and NC rats were placed in the same tank but as normal oxygen concentration environment. (2) Using echocardiography to determine left ventricular end-diastolic diameter (LVIDd), left ventricular end systolic diameter (LVIDs), left ventricular short axis shortening rate (LVFS) and left ventricular ejection fraction (LVEF) till the rats were raised at the end of the 35th day. (3) We selected samples to stain with HE, with TUNEL and with Picric acid - Sirius, then we detected the differences in myocardial structure, in myocardial apoptosis, and in the levels of myocardial fibrosis. (4)The myocardial protein was extracted to detect by Western blot comparing the expression level of HIF - 1α protein. (5) The data were analyzed by Spss 13.0. A p value less than 0.05 was considered statistically significant.

RESULTS (1) Compared with NC group, the rats of CIH group's left ventricles were dilated with expanded left ventricular internal diameter in systole (LVIDs) (4.094 ± 1.131 mm(CIH) versus 3.060±0.923mm (NC), $p < 0.01$), while left ventricular short axis shortening rate (LVFS) (38.127 ± 11.564 %(CIH) versus 51.170 ± 12.425 % (NC), $p < 0.01$) and left ventricular ejection fraction (LVEF) (74.247 ± 10.345 % (CIH) versus 87.290 ± 9.436 % (NC), $p < 0.01$)were both significantly reduced in CIH rats . (2) In CIH group, HE staining presented that myocardial cells were injured. Myocardial cell edema and part of cells necrobiosis appeared. The TUNEL positive apoptotic cells were markedly increased in the cardiac tissues of CIH-treated rats compared to control rats. Sirius staining showed a significant amount of collagen fibers in the heart of CIH rats.(3)The expression level of HIF-1α was markedly increased in the cardiac tissue of CIH rats compared to control rats by using Western blot test (0.62 ± 2.89E-0.5 (CIH) versus 0.45 ± 0.01 (NC), $P<0.05$).

CONCLUSIONS (1) CIH caused cardiac dysfunction. (2) CIH induced cardiac injuries and fibrosis. (3) CIH activated hypoxia inducible factor-1α responses, which advanced the cardiac hypoxia and inflammatory effects. Thus, the myocardial cell apoptosis and myocardial fibrosis were reflected on chronic intermittent hypoxia caused by obstructive sleep apnea.

GW26-e0803

Adipose Derived Stem Cells With Basic Fibroblast Growth Factor Improves Myocardial Repair in Rats

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OBJECTIVES To investigate whether adipose derived stem cells (ADSCs) with basic fibroblast growth factor (bFGF) could improve myocardial repair in rats.

METHODS Sixty rats with myocardial infarction (MI) were randomly divided into four groups (n=15/group): group-PBS, group-bFGF, group-ADSCs, and group-bFGF/ADSCs. Cardiac function was evaluated by echocardiogram. Bioluminescence imaging (BLI), histological analysis and immunofluorescence staining were performed to observe differentiation of ADSCs.

RESULTS The BLI signals gradually decreased in group-ADSCs and group-bFGF/ADSCs, but the signal in group-bFGF/ADSCs was constantly stronger than that in group-ADSCs ($p<0.05$). Injections of bFGF or ADSCs or bFGF/ADSCs significantly increased left ventricular ejection fraction (LVEF) compared with PBS (41.5±2.6%, 44.8±3.1%, 55.8±3.4% vs. 31.5±3.2% respectively, $p<0.01$), with implantation of bFGF/ADSC highest. The percentage of $cTnT^+/mRFP^+$ and $SMA^+/mRFP^+$ cells in group-bFGF/ADSCs was much higher than that in group-ADSCs (9.73±1.87% vs. 4.65±2.14%, 8.34±2.38% vs. 4.12±1.87% respectively, $P<0.05$). Injections of bFGF or ADSCs or bFGF/ADSCs significantly decreased apoptosis compared with PBS ($p<0.01$, respectively), with implantation of bFGF/ADSC lowest. The infarcted size was significantly reduced by the injection of bFGF/ADSCs compared with the injection of PBS (24.34% vs. 49.68%, $p<0.01$), or bFGF (36.47%, $p<0.01$), or ADSCs (33.56%, $p<0.01$). The microvessel density (MVD) in group-bFGF/ADSCs was the highest ($p<0.01$, respectively), while there was no difference in MVD between group-ADSCs and group-bFGF.

CONCLUSIONS Administration of ADSCs with bFGF could promote the growth of microvessels and improve left ventricular function and myocardial viability in the early period of MI.

GW26-e1023

Effect of β-Blocker on the Production of Collagen I in Acute Myocardial Infarction Rat

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OBJECTIVES To study the effect of carvedilol on the production of collagen and TGF-β1 in acute myocardial infarction (AMI) rat.

METHODS Coronary ligation of left anterior descending artery 40 patients with acute myocardial infarction model in rats were randomly divided into three groups: myocardial infarction control group (MI-C), a small dose of carvedilol treatment group (MI-LC 1mg/kg.d), large dose of carvedilol treatment group (MI-HC, 10 mg / kg.d). Separate the sham operation group (MI-the sham). Eight weeks later, the expressions of collagen types I (collagen I) and TGF-β1 were measured by reverse transcription-polymerase chain reaction (RT-PCR) both in infarct zone (IZ) and non-infarct zone (NIZ).

RESULTS The expressions collagen I IZ were as follow: Compared with sham rats, the expressions of collagen I MI-control rats and the other two admission groups were evidently increased ($P<0.05$). Compared with MI-control rats, the expressions were down-regulated in MI-LC rats and MI- HC rats. There were no significant differences between

MI- LC rats and MI- c HC rats (P>0.05). The expressions collagen I in NIZ was as follows: Compared with sham rats, the expressions of collagen I MI-control rats and the other two admission groups were evidently increased (P<0.05). Compared with MI-control rats, the expressions were down-regulated in MI-metoprolol rats and MI-Carvedilol rats. There were no significant differences between MI- metoprolol rats and MI- carvedilol rats (P>0.05). And also there were no differences between MI-control rats and MI-small rats (P>0.05). TGF-β1 mRNA expression is as follows: compared with the sham group (P <0.001) upregulation of TGF-β1 mRNA in the infarcted myocardium of MI rats organization. Control group and AMI in the carvedilol group, small dose of TGF-β1 mRNA is downregulated (P <0.01) in the carvedilol group, large doses of TGF-β1 mRNA levels close to the small dose of carvedilol group, two group differences were not significant (P> 0.05).

CONCLUSIONS The Carvedilol reduced the infarct zone collagen molecule synthesis and reducing the synthesis of collagen molecules in non-infarcted area, effectively retard the process of myocardial fibrosis as a whole, but no dose-dependent relationship, the mechanism may inhibit the expression of TGF-β1.

GW26-e4353

Angiotensin II Induces Atrial Fibrillation Through STAT3 Mediated Atrial Structural Remodeling

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OBJECTIVES To determine the role of angiotensin II (Ang II)/Ang II type 1 (AT1) receptor - stat3 signaling pathway in atrial structural remodeling.

METHODS Apoptosis of atrial myocytes were measured by TUNEL. Expressions of phosphorylated stat3, cytochrome C, caspase-3 and caspase-8 were measured by western blot. Transcripts of collagens and MMPs were measured by RT-PCR. The association between nuclear translocated Stat3 with MMP1 and MMP2 DNA promoter sequences was proved by CHIP.

RESULTS In atrial myocytes, incubation with AngII increased level of apoptosis, expressions of caspase 3 and 8, release of cytochrome C from mitochondria to cytosol after OGD pretreatment, which was inhibited by losartan and WP1066. In atrial fibroblasts, incubation with AngII improved transcriptions of collagenI, collagenIII, MMP1 and MMP2, which was attenuated by losartan and WP1066. In cultured atrial myocytes and fibroblasts, Ang II induced tyrosine and serine phosphorylation of STAT3, It was decreased by losartan and WP1066. STAT-3 interact with MMP1 and MMP2 DNA promoter sequences in atrial fibroblasts, the affinity was inhibited by losartan and WP1066. Rats infused with Ang II exhibited higher levels of apoptosis, collagen synthesis and phospho-STAT3 in the atria, all of which were attenuated by losartan. In human atrial tissues from patients with atrial fibrillation, levels of Ang II, percentage of apoptotic cells, synthesis of collagens and expression of phospho-STAT3 were also elevated.

CONCLUSIONS Ang II/AT1 receptor / STAT3 is an important signaling pathway in the atrial structural remodeling, Ag-II advance apoptosis of atrial parenchyma and deposition of atrial ECM, which result in atrial fibrillation.

GW26-e4720

Toll Like Receptor 9 Deficiency Attenuates Pressure Overload Induced Cardiac Remodeling

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OBJECTIVES The Innate immune system may play an important role in cardiac remodeling. Toll-like receptor 9 (TLR9) is one of the important components in the innate immune system. However, the effects and underlying mechanisms of TLR9 in cardiac remodeling are still unclear. The purpose of this study is to estimate the effects of TLR9 in cardiac remodeling and try to explore the underlying mechanisms.

METHODS First, we detected the changes of TLR9 protein level in different heart issues. Then, TLR9 (-/-) knockout (KO) mice and C57BL/6 (WT) mice (male, 8 to 10 weeks, 23.5-27.5g weight) were subjected to aortic banding (AB) operation or Sham as control. And 8 weeks after the surgery, we estimate the role of TLR9 in cardiac remodeling by echocardiography, pressure-volume (PV) detection, heart weighing, histological analysis, mRNA expression level analysis.

RESULTS The protein levels of TLR9 were up-regulated in the hearts after AB, and also ascended in human failing hearts compared with the normal donors' hearts. In the estimation of TLR9 regulating cardiac remodeling, heart weight/ body weight (HW/BW) and heart weight/ tibial length (HW/TL) were elevated after AB, but TLR9 KO mice were less obvious. Echocardiography and PV results indicated that TLR9 KO improved left ventricle systolic function and suppressed the left ventricle dilation. In the histological analysis, HE and PSR stain demonstrated that TLR9 KO reduces the degrees of cardiomyocytes hypertrophy and interstitial fibrosis. RT-PCR results showed that the mRNA levels of some hypertrophic and fibrotic markers, such as ANP, BNP, β-MHC, CTGF, TGF-β2, Collagen Ia and Collagen III, in TLR9 KO mice were raised much slightly than WT mice after AB.

CONCLUSIONS By inhibiting cardiac hypertrophy and fibrosis, TLR9 deficiency presents a protective effect on cardiac remodeling induced by pressure overload.

GW26-e0717

Lin28a Protects Against Hypoxia/Reoxygenation Induced Cardiomyocytes Apoptosis by Alleviating Mitochondrial Dysfunction Under High Glucose/ High Fat Conditions

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OBJECTIVES The aim of the present study was to investigate the role of Lin28a in protecting against hypoxia/reoxygenation(H/R)-induced cardiomyocytes apoptosis under high glucose/high fat (HG/HF) conditions.

METHODS Primary cardiomyocytes which were isolated from neonatal mouse were randomized to be treated with lentivirus carrying Lin28a siRNA, Lin28acDNA 72h before H/R (9h/2h). Cardiomyocytes biomarkers release (LDH and CK), cardiomyocytes apoptosis, mitochondria biogenesis and morphology, intracellular reactive oxygen species (ROS) production, ATP content and inflammatory cytokines levels after H/R injury in high glucose/high fat conditions were compared between groups. The target proteins of Lin28a were examined by western blot analysis.

RESULTS Our results revealed that Lin28a cDNA transfection (overexpression) significantly inhibited cardiomyocyte apoptotic index, improved mitochondria biogenesis, increased ATP production and reduced ROS production as compared with the H/R group in HG/HF conditions. Lin28a siRNA transfection (knockdown) rendered the cardiomyocytes more susceptible to H/R injury as evidenced by increased apoptotic index, impaired mitochondrial biogenesis, decreased ATP production and increased ROS level. Interestingly, these effects of Lin28a were blocked by pretreatment with the PI3K inhibitor wortmannin. Lin28a overexpression increased, while Lin28a knockdown inhibited IGF1R, Nrf-1,Tfam, p-IRS-1, p-Akt, p-mTOR, p-p70s6k, p-AMPK expression levels after H/R injury in HG/HF conditions. Moreover, pretreatment with wortmannin abolished the effects of Lin28a on the expression levels of p-AKT, p-mTOR, p-p70s6k, p-AMPK.

CONCLUSIONS The present results suggest that Lin28a inhibits cardiomyocytes apoptosis by enhancing mitochondrial biogenesis and function under high glucose/high fat conditions. The mechanism responsible for the effects of Lin28a is associated with the PI3K/Akt dependent pathway.

GW26-e1481

Sphingosine Kinase-2 Protects Against Myocardial Infarction-induced Heart Failure by Inhibiting Histone Deacetylase Activation

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OBJECTIVES Myocardial infarction (MI) is the leading cause of heart failure. Persistent histone deacetylase (HDAC) activation in the myocardium contributes to heart failure progression after MI. Sphingosine kinase-2 (SphK2) and its product sphingosine 1-phosphate (S1P) have been recognized as endogenous inhibitors of HDAC. However, the role of SphK2 in the progression of post-MI heart failure remains unknown.

METHODS MI models were induced by permanent coronary artery ligation in adult C57bl6 mice. After the operation, ABC294640 (ABC), a specific SphK2 inhibitor, was administrated intraperitoneally daily or until the animal died. The survival conditions of operated-mice were monitored daily. Left ventricular ejection function was measured by echocardiography and cardiac remodeling was evaluated by Masson's